(6) Arsenic—Transfer 1.0 g of L-Methionine to a 100-mL decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add two 2-mL portions of nitric acid, heat, add 2-mL portions of hydrogen peroxide (30) several times, and heat until the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution using Apparatus B (not more than 2 ppm).

(7) Other amino acids—Dissolve 0.10 g of L-Methionine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

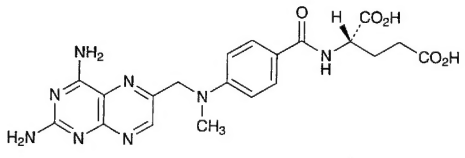
Assay Weigh accurately about 0.15 g of L-Methionine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.921 mg of $C_5H_{11}NO_2S$

Containers and storage Containers—Tight containers.

Methotrexate

メトトレキサート



$C_{20}H_{22}N_8O_5$: 454.44
N-{4-[*N*-(2,4-Diaminopteridin-6-ylmethyl)-*N*-methylamino]-benzoyl}-L-glutamic acid [*59-05-2*]

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds. It contains not less than 94.0% and not more than 102.0% of $C_{20}H_{22}N_8O_5$, calculated on the anhydrous basis.

Description Methotrexate occurs as a yellow-brown, crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetonitrile, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

Identification **(1)** Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methotrexate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methotrexate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Methotrexate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with water determination TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

Residue on ignition Not more than 0.10% (0.5 g).

Assay Weigh accurately about 0.025 g each of Methotrexate and Methotrexate Reference Standard, dissolve each, in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and measure the peak areas, A_T and A_S, of methotrexate in each solution.

Amount (mg) of $C_{20}H_{22}N_8O_5$
= amount (mg) of Methotrexate Reference Standard, calculated on the anhydrous basis
$\times \frac{A_T}{A_S}$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of disodium hydrogenphosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

Selection of column: Dissolve 0.010 g each of Methotrexate and folic acid in 100 mL of the mobile phase.

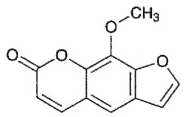
Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of folic acid and methotrexate in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 2.5%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Methoxsalen

メトキサレン



$C_{12}H_8O_4$: 216.19
9-Methoxy-7*H*-furo[3,2-*g*]chromen-7-one
[*298-81-7*]

Methoxsalen contains not less than 98.0% and not more than 102.0% of $C_{12}H_8O_4$, calculated on the anhydrous basis.

Description Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification **(1)** To 0.01 g of Methoxsalen add 5 mL of dilute nitric acid, and heat: a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

(2) To 0.01 g of Methoxsalen add 5 mL of sulfuric acid, and shake: a yellow color develops.

(3) Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (1 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methoxsalen Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point 145 - 149°C

Purity **(1)** Heavy metals—Proceed with 1.0 g of Methoxsalen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Methoxsalen according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(3) Related substances—Dissolve 0.050 g of Methoxsalen in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, hexane and ethyl acetate (40:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water Not more than 0.5% (1 g, direct titration).

Residue on ignition Not more than 0.10% (1 g).

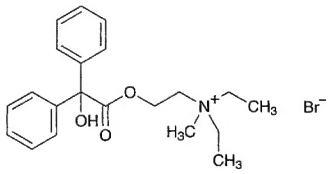
Assay Weigh accurately about 0.05 g each of Methoxsalen and Methoxsalen Reference Standard, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 300 nm as directed under the Ultraviolet-visible Spectrophotometry.

Amount (mg) of $C_{12}H_8O_4$
= amount (mg) of Methoxsalen Reference Standard, calculated on the anhydrous basis
$\times \frac{A_T}{A_S}$

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Methylbenactyzium Bromide

臭化メチルベナクチジウム



$C_{21}H_{28}BrNO_3$: 422.36
N,*N*-Diethyl-*N*-[2-(hydroxydiphenylacetoxy)ethyl]-*N*-methylammonium bromide [*3166-62-9*]

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of $C_{21}H_{28}BrNO_3$.

Description Methylbenactyzium Bromide occurs as white crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Methylbenactyzium Bromide (1 in 50) is between 5.0 and 6.0.